

## On the identity of the so-called "algae like cells" in tadpole cultures of European green frogs (*Rana ridibunda*)

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Green algae of the genus *Chlorella*, corresponding morphologically to the so-called "algae like cells" of previous authors, were found in laboratory water conditioned with tadpoles of European Green Frogs. The colour of these cells seems to depend on the light conditions under which the tadpoles are raised. These algae were found in varied culture conditions, including those where the tadpoles show rapid and synchronized development, this suggests that these algae do not play a significant inhibitory role in the development of tadpoles.

### INTRODUCTION

In the course of studying the micro-organisms associated with the alimentary tract of tadpoles of European Green Frogs (*Rana ridibunda* of hybrid origin), I found the same cellular types that had been described and photographed by RICHARDS (1958, 1962), AKIN (1966), LICHT (1967), and HEUSSER & BLANKENHORN (1973), cultured from other tadpoles of various American and European anurans. Some of these authors attributed an inhibitory role to these cells during the growth of larvae. This paper is devoted to their identification, with a comment on this proposed inhibitory role.

### MATERIAL AND METHODS

The larvae studied came from a clutch deposited the night of 5 June 1985 in the laboratory by a pair of diploid *Rana* kl. *esculenta* specimens, which had been found in amplexus the same day in a pond in the Fontainebleau forest near Paris. These tadpoles were *Rana ridibunda* of hybrid origin (GRAF & POLLS, in prep.). They were reared in the Paris Museum from June to September 1985 at various densities (5-30 tadpoles/liter), volumes of water (0,5-1,5 liter), and exposed to varied intensities and sources (natural and artificial) of light. The culture water was tap water which had remained standing for 24-28 hours for dechlorination; it was replaced in part (1/3 of each volume) every 1-2 days. The tadpoles were fed "ad libitum" with a progressive diet of boiled lettuce salad, Tetramin, and cooked egg

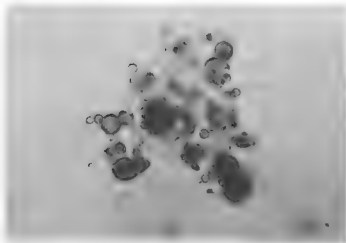


Fig. 1 *Chlorella* green algae cells found in tadpoles' cultures, corresponding to the so-called "algae like cells" of previous authors.

yolk. Microscopic observations, measurements and photographs were made at magnification of 400. Initial observations were made just after sampling, with samples isolated in sterilized 20 cm<sup>3</sup> flasks, under natural North-oriented sun-light. Later observations were made on the same samples, from a week to several months after isolation. Taxonomic identification of the algae cells was confirmed by Dr. BOURRELY.

## RESULTS

The same cell types that had previously been described in the American and European literature (see above) were found in all water samples – with, or without, any inhibition on growth of the tadpoles. The colour of these cells was variable ; cells from the samples with natural light were green, while those from the samples with weak artificial light were uncoloured. Independent of colour, all these cells had the morphological characteristics of green algae belonging to the genus *Chlorella*. The cells often formed aggregations (fig. 1). The characteristics were the same as described by BOURRELY (1966) for all of probably more than 30 species in the genus :

- Spherical or elliptic cells.
- Cells containing a plast and a pyrenoid.
- Diameter from few to maximal 20 micrometers.

After one week of incubation of the uncoloured forms under strong sun-light and in isolation from external contamination, major blossom of green *Chlorella* cells was observed, with an almost total disappearance of the non-coloured forms. The same bottles, put in total darkness, retained green algae for several months.

## DISCUSSION

RICHARDS (1962) tentatively identified the same types of algae, which she found uncoloured, as belonging to the genus *Prototheca*. The morphology of the two genera is very similar, and they only differ with respect to colour – green (*Chlorella*), versus unpigmented (*Prototheca*); however, some mutations are known in the genus *Chlorella* which affect the number of pigment molecules (MARGALEF, 1983). The possibility exists that there are two algal genera in my samples, but this would not alter any conclusions about the possible inhibitory role of these cells in tadpoles growth. It should be noted that, in the same culture water where I found *Chlorella*, several species of algae (e.g. *Selenastrum*, *Euglena*, *Phacus*, *Cosmarium*, *Scenedesmus*, *Phacotus*), protozoa and bacteria (e.g. *Zooglea*, *Moraxella*, *Aeromonas*, *Kurthia*, *Micrococcus*) were also found (KAISER & POLLS, in prep.).

AKIN (1966) suggested that the “algae like cells” could execute an inhibitory role on tadpoles as carriers of a molecule present in the intestine of anuran larvae. Following the opinions of WEST (1960) and STEINWASCHER (1978) – who discusses earlier accounts by ROSE & ROSE (1961), STEPANOVA (1974) and RUNKOVA, STEPANOVA & KOVAL'CHUK –, I consider that, except possibly at extremely high concentrations, *Chlorella* plays a negligible role in the initiation of growth inhibition.

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## RÉSUMÉ

Au cours d'un élevage de têtards de la Grenouille verte européenne, des algues vertes appartenant au genre *Chlorella* ont pu être détectées; leur morphologie était identique à celle décrite par d'autres auteurs dans d'autres cultures de têtards, pour d'autres espèces d'Anoures dans d'autres localités; à cause de leur caractéristique incolore, ces algues avaient été appelées des “algae like cells”. Dans nos élevages la présence des cellules vertes (*Chlorella*) ou des non colorées paraît dépendre des conditions d'éclairage. Des *Chlorella* et des cellules non colorées ont été retrouvées dans différents cristallisoirs d'élevage des têtards, y compris ceux où on a observé un développement bon et rapide, synchronisé pour le groupe.

De ce point de vue, le rôle inhibiteur de la croissance des têtards, attribué par d'autres auteurs à ces algues, ne semble pas devoir seulement dépendre de la présence qualitative des “algae like cells” ou des *Chlorella*.

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